A consolidated linkage map for rainbow trout (*Oncorhynchus mykiss*)

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Summary

Androgenetic doubled haploid progeny produced from a cross between the Oregon State University and Arlee clonal rainbow trout (*Oncorhynchus mykiss*) lines, used for a previous published rainbow trout map, were used to update the map with the addition of more amplified fragment length polymorphic (AFLP) markers, microsatellites, type I and allozyme markers. We have added more than 900 markers, bringing the total number to 1359 genetic markers and the sex phenotype including 799 *Eco*RI AFLPs, 174 *Pst*I AFLPs, 226 microsatellites, 72 VNTR, 38 SINE markers, 29 known genes, 12 minisatellites, five RAPDs, and four allozymes. Thirty major linkage groups were identified. Synteny of linkage groups in our map with the outcrossed microsatellite map has been established for all except one linkage group in this doubled haploid cross. Putative homeologous relationships among linkage groups, resulting from the autotetraploid nature of the salmonid genome, have been revealed based on the placement of duplicated microsatellites and type I loci.

Keywords amplified fragment length polymorphism, microsatellites, *Oncorhynchus mykiss*, type I markers.

Introduction

Rainbow trout (*Oncorhynchus mykiss*) are cultured widely throughout the world for supplementation in natural ecosystems and for commercial aquaculture. This wide distribution is facilitated by the great phenotypic variability and range of adaptations exhibited among populations (Hershberger 1992). Two molecular genetic linkage maps for rainbow trout (Young *et al.* 1998; Sakamoto *et al.* 2000) and one composite salmonid allozyme map (May & Johnson 1990) have been developed. The more recent molecular maps have provided the foundation for genetic analysis of some of these traits, including development rate (Robison *et al.* 2001), upper thermal tolerance (Perry *et al.* 2001), disease resistance (Ozaki *et al.* 2001), albinism (Nakamura *et al.* 2001) and spawning date (Sakamoto *et al.* 1999).

The salmonid fishes are thought to be derived from a common tetraploid ancestor, which arose by autotetraploidy 25–100 Mya (Allendorf & Thorgaard 1984). This theory is supported by consistent numbers of chromosome arms in karyotypes of most salmonids and by the prevalence of duplicated enzyme and gene loci. Most salmonids have 100–104 chromosome arms (Allendorf & Thorgaard 1984), which is approximately twice the number of chromosome arms observed in many of the teleosts (i.e. 48–50 one-armed chromosomes; Denton 1973). For the most part, disomic inheritance has been restored in the salmonids, but residual tetrasomy has been observed, as evidenced by inheritance patterns and by the presence of multivalents (pairing of both

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homologous and homeologous chromosome arms) in meiotic preparations (Johnson *et al.* 1987; Allendorf & Danzmann 1997). Although the tetraploid ancestry and varying degrees of tetrasomy pose particular challenges for linkage mapping in salmonid fishes, signatures of these phenomena, observed with mapping of duplicated loci, can identify ancient chromosome homeologues in this group of fishes.

Our previously published rainbow trout map (Young *et al.* 1998) has provided the framework for genetic analysis of complex traits in clonal lines of rainbow trout (Robison *et al.* 2001). These clonal lines of rainbow trout have been derived from hatchery and natural populations with unique developmental, physiological and other life history characteristics of evolutionary significance. The use of inbred or clonal lines facilitates molecular marker screening and genetic analysis of quantitative traits. The system that we use for propagating trout by manipulation of gametes and zygotes has enabled the production of doubled haploid progeny for linkage mapping and quantitative trait locus analyses. Dominant marker systems such as amplified fragment length polymorphic (AFLP) markers can be scored without ambiguity in doubled haploid progeny, as all individuals are homozygous. The AFLP systems have provided numerous markers with less cost and time than other marker systems, and have provided the framework upon which other marker types have been added.

This map update consolidates the existing *O. mykiss* linkage maps (May & Johnson 1990; Young *et al.* 1998; Sakamoto *et al.* 2000) and will serve as a roadmap for future quantitative and molecular genetic analyses. We have added AFLP, microsatellite and known gene markers to improve marker coverage and link existing published maps. To establish synteny with other salmonid maps, microsatellites mapped in an outbred *O. mykiss* cross (Sakamoto *et al.* 2000) and allozyme loci in a composite salmonid map (May & Johnson 1990) have been added to this update.

Materials and methods

Mapping progeny

The doubled haploid rainbow trout used to construct our original linkage map (Young *et al.* 1998) were used for the addition of molecular and allozyme markers to this updated map. These progeny were produced from the F1 hybrid from a cross between the Oregon State University (OSU) and Arlee (ARL; Arlee, Montana) isogenic lines (Young *et al.* 1996). Doubled haploids were produced by androgenesis, as described by Young *et al.* (1998). Briefly, eggs from outbred females were irradiated with gamma radiation to destroy maternal nuclear DNA and were then fertilized with sperm from an OSU × ARL hybrid F1. The first embryonic cleavage was blocked with a heat shock to restore diploidy, resulting in doubled haploid individuals with all-paternal nuclear DNA inheritance. Tissues were taken from these individuals and extracted as described by Young *et al.* (1998).

Molecular marker genotyping

Methods for markers previously mapped are detailed in Young *et al.* (1998). The following methods refer to markers added for this linkage map update.

Amplified fragment length polymorphism: Amplified fragment length polymorphic marker protocols followed those of Vos *et al.* (1995). The restriction enzymes *Pst*I or *Eco*RI were used with *Mse*I to digest genomic DNA for selective amplification. *Pst*I or *Eco*RI adapter primers were labelled with either Cy5 or ^{33}P and products separated on 5% denaturing polyacrylamide gels were visualized by fluorescence imaging (Robison *et al.* 2001), or autoradiography (Young *et al.* 1998), respectively. Polymorphic markers were named to designate the six-cutting enzyme, +3 selective bases, marker size and presence in parents. The first letter corresponds to the six-cutter restriction enzyme used, with E designating *Eco*RI and P for *Pst*I. The first triplet of letters represents the *Eco*RI or *Pst*I adapter +3 selective bases and the second triplet corresponds to the *Mse*I adapter +3 selective bases. The number corresponds to the approximate size in base pairs of the fragment and the letter following corresponds to the parent in which the band was present. The letter 'o' denotes presence in the OSU parent, 'a' presence in ARL and 'c' represents a codominant marker. For example, *Eaaccta450o* represents a 450-bp fragment that is present in the OSU line, and was detected using the *Eco*RI aac and the *Mse*I cta selective primers.

Microsatellites: Both new and old microsatellite primers developed for salmonids were used for genotyping in this study. Primer names are informative for both the species in which the microsatellite was developed (Table 1), and the laboratory and references in which primer design is detailed (Table 2). Genotypes were scored on agarose when size differences were large enough, or were scored by fluorescence detection platforms using polyacrylamide gel electrophoresis when allele sizes were too close to discriminate

Table 1 Microsatellite species abbreviations.

Acronym	Scientific name	Common name
Ocl	*Oncorhynchus clarki*	Cutthroat trout
Ogo	*Oncorhynchus gorbuscha*	Pink salmon
Oke	*Oncorhynchus keta*	Chum salmon
Oki	*Oncorhynchus kisutch*	Coho salmon
Omy, OMM	*Oncorhynchus mykiss*	Rainbow and steelhead trout
One	*Oncorhynchus nerka*	Sockeye salmon
Ots, Ot	*Oncorhynchus tshawytscha*	Chinook salmon
Sal	*Salvelinus alpinus*	Arctic char
SS, Ssa	*Salmo salar*	Atlantic salmon
Str	*Salmo trutta*	Brown trout

Table 2 Microsatellite source acronyms.

Acronym	Laboratory	Reference(s)
ADFG	Alaska Department of Fish and Game, Anchorage, AL, USA	Olsen *et al*. (2000)
ASC	Alaska Science Center, Anchorage, AL, USA	Scribner *et al*. (1996)
BML	Bodega Marine Laboratory, University of California, Bodega Bay, CA, USA	Banks *et al*. (1999)
DIAS	Danish Institute of Agricultural Science, Tjele, Denmark	Holm & Brusgaard (1999)
DU	Dalhousie University, Halifax, Nova Scotia, Canada	Morris *et al*. (1996)
INRA	Institut National de la Recherche Agronomique, Jouy-en-Josas, France	K. Gharbi and R. Guyomard (pers. comm.)
LEE	National Fish Health Research Laboratory, Leetown, WV, USA	W. B. Schill and Walker (pers. comm.)
NUIG	National University of Ireland, Galway	R. Powell (pers. comm.)
NVH	Norwegian College of Veterinary Medicine, Oslo, Norway	B. Hoyheim (pers. comm.)
NWFSC	North-west Fisheries Science Center, NOAA-National Marine Fisheries Service, Seattle, WA, USA	K. Naish (pers. comm.)
OMM	National Center for Cool and Cold Water Aquaculture, USDA-ARS, Leetown, WV, USA	Rexroad *et al*. (2001, a, b, c))
OSL	Norwegian College of Veterinary Medicine, Oslo	Slettan *et al*. (1997)
SSBI	SeaStar Biotech Incorporated, Victoria, BC, Canada	Nelson & Beacham (1999) Small *et al*. (1998)
TUF	Tokyo University of Fisheries, Japan	Sakamoto *et al*. (1996) Khoo *et al*. (2000a, b) T. Sakamoto & N. Okamoto (pers. comm.)
UoG	University of Guelph, Canada	Jackson *et al*. (1998)
UW	University of Washington, School of Fisheries, Seattle, WA, USA	Condrey & Bentzen (1998) Olsen *et al*. (1998) P. Bentzen (pers. comm.)

on agarose. A total of 108 of these microsatellites have been previously mapped in another rainbow trout cross (Sakamoto *et al*. 2000) and were mapped here to identify syntenic relationships among the maps. Some microsatellite markers were scored independently by at least two different labs. In this case, a composite was made by combining the data from the independent data sets. If genotypes were discordant for an individual, that individual was given a missing genotype. The markers for which composites were made are: *One2ASC*, *One11ASC*, *Ots521NWFSC and Ots100SSBI*.

Type I loci: References for gene-specific primer development and amplification parameters are found in Table 3. Polymerase chain reaction products were visualized on 1.5–2% agarose stained with ethidium bromide to identify successful amplicons. For 5′*ETS*, *CBR1*, *FGF6*, *GH1*, *GH2*, *MT1B* and *NRAMP-alpha*, polymorphisms were visualized by the single strand conformation polymorphism technique (Vidal-Puig & Moller 1994). For all other loci, the gene was sequenced and polymorphisms were identified by sequence alignment of OSU and ARL parents. Marker polymorphisms identified by sequence alignment were genotyped by restriction fragment length polymorphism (RFLP), insertion/deletion (indel) polymorphisms, or by detection of single nucleotide polymorphisms (SNP). In cases where both OSU and ARL alleles possessed restriction sites, each site was used in reciprocal tests for the confirmation of genotype. The RFLPs were visualized on 1.5–3% agarose stained with ethidium bromide. ITIM-bearing C type lectin (*TCL-2*) and *TCRB@* alleles were detected by a size difference on agarose (Zhang *et al*. 2001). All other indel polymorphisms were smaller in size and were targeted by design of forward primers specific for the insertion or deletion of the two alleles. *HRAS* and *SOD1* were amplified with both the ARL and OSU specific forward primers and the same reverse primers separately, and selective amplification was detected on agarose. *WT1-a* and *WT1-b* were sequenced with both forward and reverse primers to detect the identity of the SNP allele (Brunelli *et al*. 2001). All other SNPs were detected with the ABI SNaPshot dideoxynucleotide terminating protocol (Applied Biosystems International, Foster City, CA, USA) with primers designed to target the SNP.

Allozymes: Horizontal starch gel electrophoresis was used to determine the genetic characteristics of each fish at allozyme loci coding for proteins present in muscle or liver tissue (Leary 1997). Electrophoresis followed the procedures of Allendorf & Utter (1979) and Leary & Booke (1990). Gel buffers and stains used to reveal the position of particular enzymes in the gels after electrophoresis followed the protocols of Allendorf *et al*. (1977). Loci are named according to the American Fisheries Society standard (Shaklee *et al*. 1990). All but four loci screened were monomorphic between the OSU and ARL clonal lines. Polymorphic allozymes typed for mapping were malate dehydrogenase 1

Table 3 Genes mapped in doubled haploid mapping panel. See text for details on genotype detection.

Acronym	Gene	Genotype detection	GenBank accession	Citation
5′ETS	External transcribed spacer for 18S/28S rDNA	SSCP	Z18683	Le *et al.* (1993)
CBR1	Carbonyl reductase	SSCP	AF100933	Guan *et al.* (1999)
CTSD	Cathepsin D	SNP	U90321	Moran (2002)
FGF6	Fibroblast growth factor 6	SSCP	Y16850	Rescan (1998)
GH1	Growth hormone 1	SSCP	AF005923	Oakley & Phillips (1999)
GH2	Growth hormone 2	SSCP	M22732	Agellon *et al.* (1988)
GTH2B	Gonadotropin hormone II beta subunit	RFLP-*SspI*	L17140, M34850	Baker (2001)
HRAS	Ras-1 oncogene	in/del	M73690	Moran (2002)
ID1	Inhibitor of DNA binding/differentiation 1	SNP	Y08368	Moran (2002)
MHC1-TAP2B	Major histocompatability complex I transport and activating protein 2B	RFLP-*HindIII*	AF115538	Hansen *et al.* (1999)
MT1B	Metallothionein B	SSCP	M22487	Zafarullah *et al.* (1988)
MYC	C-myc proto-oncogene	RFLP-*TaqI*	S79770	Panno & McKeown (1995)
NRAMP-alpha	Natural resistance associated macrophage protein alpha	SSCP	AF048760	Dorschner & Phillips (1999)
PRL	Prolactin	SNP	M24738	
RAG1	Recombination activation gene-3′ UTR	SNP	U73750	Baker (2001)
RB1	Retinoblastoma	RFLP-*MseI/BstNI*	AF102861	Brunelli & Thorgaard (1999)
SOD1	Cu/Zn-superoxide dismutase 1	in/del	AF469663	
Somatolactin	Somatolactin	SNP	AF223839	Moran (2002)
TCL-1	C-type lectin	RFLP-*AluI*		Zhang *et al.* (2000)
TCL-2	ITIM-bearing C-type lectin	in/del		Zhang *et al.* (2001)
TP53	p53 proto-oncogene	RFLP-*AluI*	M75145	de Fromentel *et al.* (1992)
TRB@	T-cell receptor beta	In/del	AF329700	H. Zhang & Bistow unpublished data
TRCARR/i *TRCARR/ii*	Trout red cell arrestin	SNP		Sakamoto *et al.* (2000)
UQCRCI	Ubiquinol-cytochrome c reductase core I protein	RFLP-*Tsp509I*	AF465782	
VIM	Vimentin	SNP	Z50738	Moran (2002)
WT1-a	Wilms tumour – type 1a	SNP	AF334670	Brunelli *et al.* (2001)
WT1-b	Wilms tumour – type 1b	SNP	AF334671	Brunelli *et al.* (2001)
ZNFN1A1	Zinc finger protein, subfactor 1 A, 1 (Ikaros)	SNP	U92199-201	Moran (2002)

(sMDH-B1,2, IUPAC 1.1.1.37), b-N-acetylhexosaminidase (bGLUA, IUPAC 3.2.1.52), isocitrate dehydrogenase 1 (sIDHP-1,2, IUPAC 1.1.1.42) and phosphoglycerate kinase (PGK-2, IUPAC 2.7.2.3). These allozymes were mapped in the first published linkage map for salmonids (May & Johnson 1990) and were typed in our cross in order to evaluate synteny between published maps.

Statistical analyses

Segregation distortion: As a diagnostic test for marker reliability, all markers were tested for significant deviation from expected Mendelian segregation ratios using a chi-square test prior to linkage analysis ($\alpha = 0.05$). Markers that deviated significantly from Mendelian ratios were re-checked for reliability and scoring errors, and were removed if unreliable. Distorted markers that were retained for linkage analysis are denoted with '^' for loci with a greater than expected number of OSU genotypes and with '*' for a greater than expected number of ARL genotypes.

Linkage analysis: Genotype data from newly scored markers were appended to genotype data from our published map (Young *et al.* 1998). The Kosambi map function was used for linkage analysis, as salmonids have exhibited crossover interference (Thorgaard *et al.* 1983). To facilitate marker ordering, large marker clusters were initially identified using a LOD of 10 and minimum distance of 0 cM using Mapmanager (Manly & Olson 1999). For each cluster identified in initial and subsequent analyses (LOD > 3.0, $\theta < 1.0$ cM), the marker with the greatest number of progeny genotyped was chosen as the representative for the cluster. In subsequent analyses, genotype data from all but the representative were removed from the data file for marker grouping and ordering. Mapmaker for Mac v. 2.0 (Dr Scott Tingey, Dupont Experimental Station, Wilmington, DE, USA) was used for initial grouping of markers using the doubled haploid cross (minimum LOD 3.0, maximum θ of 35.0 cM). Smaller groups identified with these parameters were ordered using the 'compare' and 'try' commands. Large groups that were not ordered at LOD 3 were subsequently broken down by increasing the minimum LOD

to 4.0 and then to 5.0, and then ordered in the same way. Initial marker ordering was completed by at least two people independently. Additional clustered markers identified at lower LOD scores were checked for identity to their representative and removed from subsequent analyses. Initial marker orders were checked using the 'ripple' command in Mapmaker/EXP (Lander *et al*. 1987) with the window size set to six markers and order discrepancies were subsequently tested by 'compare' within the unquestioned framework of markers. Potential marker errors were further identified with the error detection and 'geno' functions in Mapmaker/EXP. Markers that expanded map distance were double-checked for errors, and if deemed unreliable, were removed from the analysis. With a framework set from the above analyses, markers that were excluded from groups with more stringent LOD scores were assigned to linkage groups at a LOD of 3.0. Once all markers had been placed in this framework, groups were 'rippled' once more to confirm the best order, and ambiguous areas were re-ordered using 'compare' within the framework of unambiguously ordered markers. Final map distances were calculated using Mapmaker for Mac.

Marker coverage: The overall distribution of marker types across linkage groups was compared by standardizing the size of each linkage group and dividing them into eight equal sized bins. Bins 1 and 8 represent the distal ends of a linkage group, while the central bins represent the center of the linkage group. The location of the largest cluster on each linkage group is assumed to be the approximate location of the centromere. Centromeres were assigned to the same bins based on placement along the linkage group for comparison to marker distributions.

Evidence for clustering of *Eco*RI and *Pst*I AFLP, and microsatellite markers was analysed based on the null expectation that markers were distributed across the 600 unique map positions in a Poisson manner (where $P(x) = e^{-\mu}\mu^{x}/x!$; μ = mean number of markers expected at each unique map position under a Poisson distribution, x = the number of markers observed at each unique map position: $\mu_{EcoRI} = 1.325$, $\mu_{PstI} = 0.295$, $\mu_{micro} = 0.345$). Clusters for this statistical analysis were defined as any unique map position that was represented by more than one of the particular marker type in question. We used a 0-cM interval as the cluster boundaries, which is a more conservative definition of a cluster than that in a similar analysis by Young *et al*. (1999). Only marker classes (AFLP and microsatellite) for which large numbers were added to this update ($n > 100$) were tested for non-random distribution.

Results

Linkage map overview

Thirty major linkage groups were delineated with the addition of new markers in the OA mapping panel (Fig. 1). The major linkage groups are characterized by greater than 20 markers with a large number of clustered markers in one region of each linkage group. In addition to these major linkage groups, three smaller groups consisting of eight or fewer markers and seven marker pairs were observed. The markers in eight of these 10 smaller groups, when 'assigned' in Mapmaker to the framework of larger groups, were assigned to single groups at a minimum LOD of 3.0. However, when these markers were placed in the framework linkage groups to which they were assigned, they could not be placed within 40 cM of the outermost markers of the linkage groups, and thus greatly expanded the map distance. OA-XXVIII in Fig. 1, was depicted as a major linkage group in Young *et al*. (1998), but with this map update absorbed only one additional marker (*Etccctt470a*). This linkage group lacks the clustering characteristic of the major groups and thus is no longer considered a major linkage group representative of an entire physical chromosome.

The current map data consists of 1359 genetic markers and the sex phenotype. The genetic markers include 799 *Eco*RI AFLPs, 174 *Pst*I AFLPs, 226 microsatellites, 72 VNTR, 38 SINE markers (*Hpa*/*Fok*), 29 known genes, 12 minisatellites, five RAPDs, and four allozymes. The 30 major linkage groups together hold 1314 of these markers, while the remaining markers are found on the smaller groups ($n = 30$) or are unlinked ($n = 15$). Twenty-six to 78 doubled haploids used for linkage mapping in Young *et al*. (1998) were genotyped for the added markers, with an average of 55 individuals genotyped for the markers in the updated data file. This average of 55 informative meioses gives an average map resolution of 1.8 cM. The genotype data for all markers, and information about AFLP clusters and microsatellite and type I locus primer sequences and GenBank accession numbers can be found at http://www.wsu.edu/~thorglab/OAmapupdate.html.

Marker coverage

In total, the 40 linkage groups cover 4590 cM of the genome. The 30 major linkage groups total 4359 cM. Except for one group consisting of a pair of markers, all small groups identified by Young *et al*. (1998) were linked to the major linkage groups in this map update. The average intermarker distance for the 30 major groups, counting only non-zero cM distances, is approximately 7.4 cM (4359 cM/593 unique map positions).

Marker types were distributed non-uniformly across the map. The AFLP, microsatellite and gene markers tended to map more frequently to the center of linkage groups, while minisatellite/VNTR type markers were more often found towards the ends of the linkage groups. Under the null hypothesis that markers are distributed randomly throughout the genome, the number of markers at each map position is expected to follow a Poisson distribution.

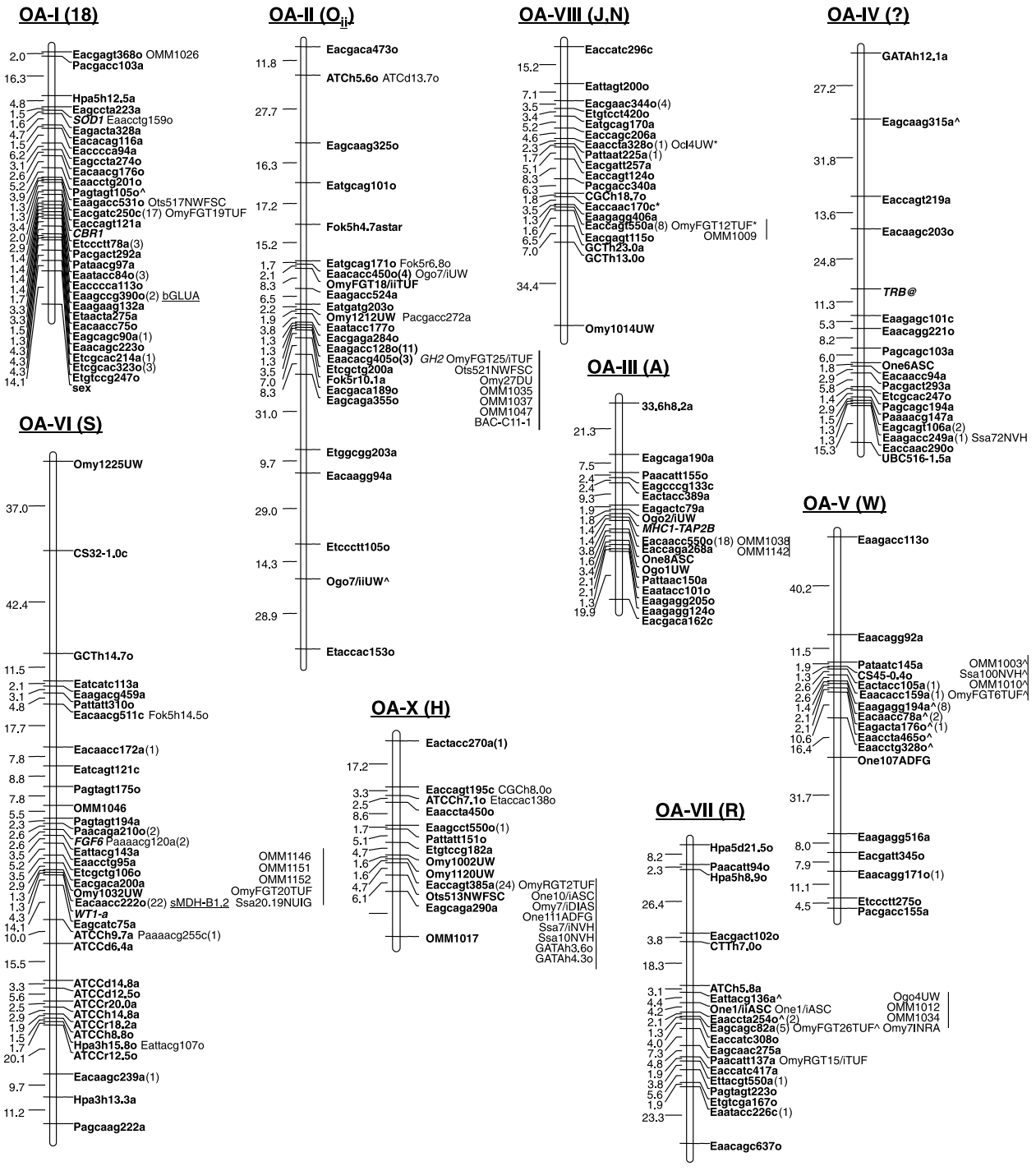

Figure 1 OA linkage groups with centiMorgan (cM) distances on the left and markers on the right. Characters in parentheses following OA designation are Sakamoto *et al*. (2000) linkage groups showing synteny. Markers in bold were used for linkage analysis statistics, and are the representatives of clustered markers that are not in bold. Numbers in parentheses following AFLP marker names indicate the number of additional AFLP markers found in that cluster. Lines to the right of a group of markers indicate membership in the same cluster. Type I loci names are italicized, allozymes are underlined. Markers exhibiting significant deviation from expected Mendelian segregation ratios are denoted with '^' if a greater number of OSU genotypes were observed and with '*' if a greater number of Arlee genotypes were observed.

*Eco*RI AFLP ($\chi^2 = 672.5$, 6 d.f.; $P < < 0.0001$), *Pst*I AFLP ($\chi^2 = 704.2$, 4 d.f.; $P < < 0.0001$), and microsatellite markers ($\chi^2 = 793.8$, 4 d.f.; $P < < 0.0001$) differed significantly from the Poisson expectation. The proportion of each class of AFLP markers existing in clusters was not significantly different ($\chi^2 = 0{\cdot}045$, 1 d.f.; $P = 0.833$).

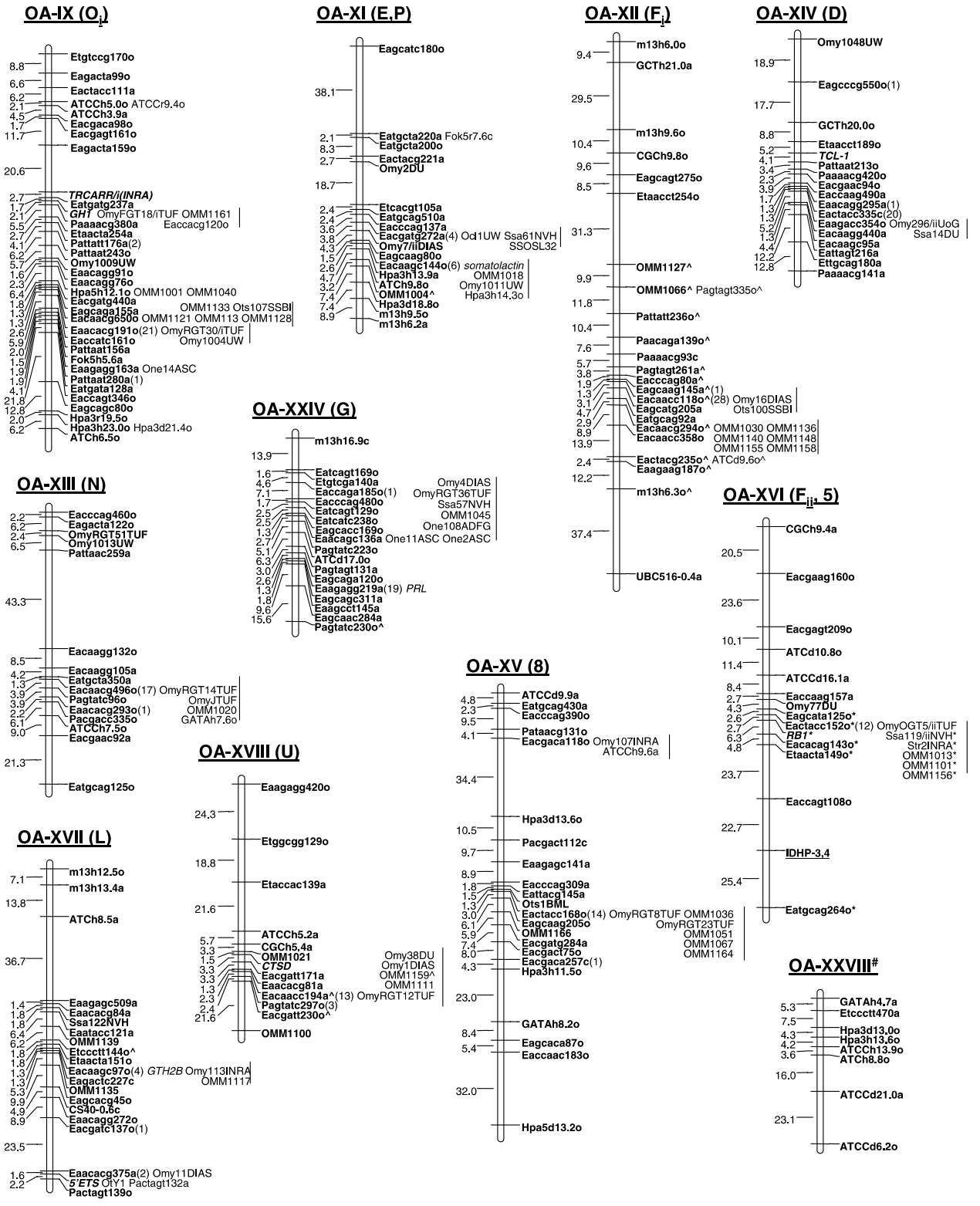

Figure 1 Continued.

Known gene markers were localized to 20 of the 30 major linkage groups. Of special note, *SOD1*, bGLUA (formerly HEX) and *CBR1* mapped to the sex chromosome. As found previously by Allendorf *et al.* (1994), bGLUA is located near the large cluster of markers on the sex chromosome, and is closer to the sex locus than *SOD1*, which is located on the

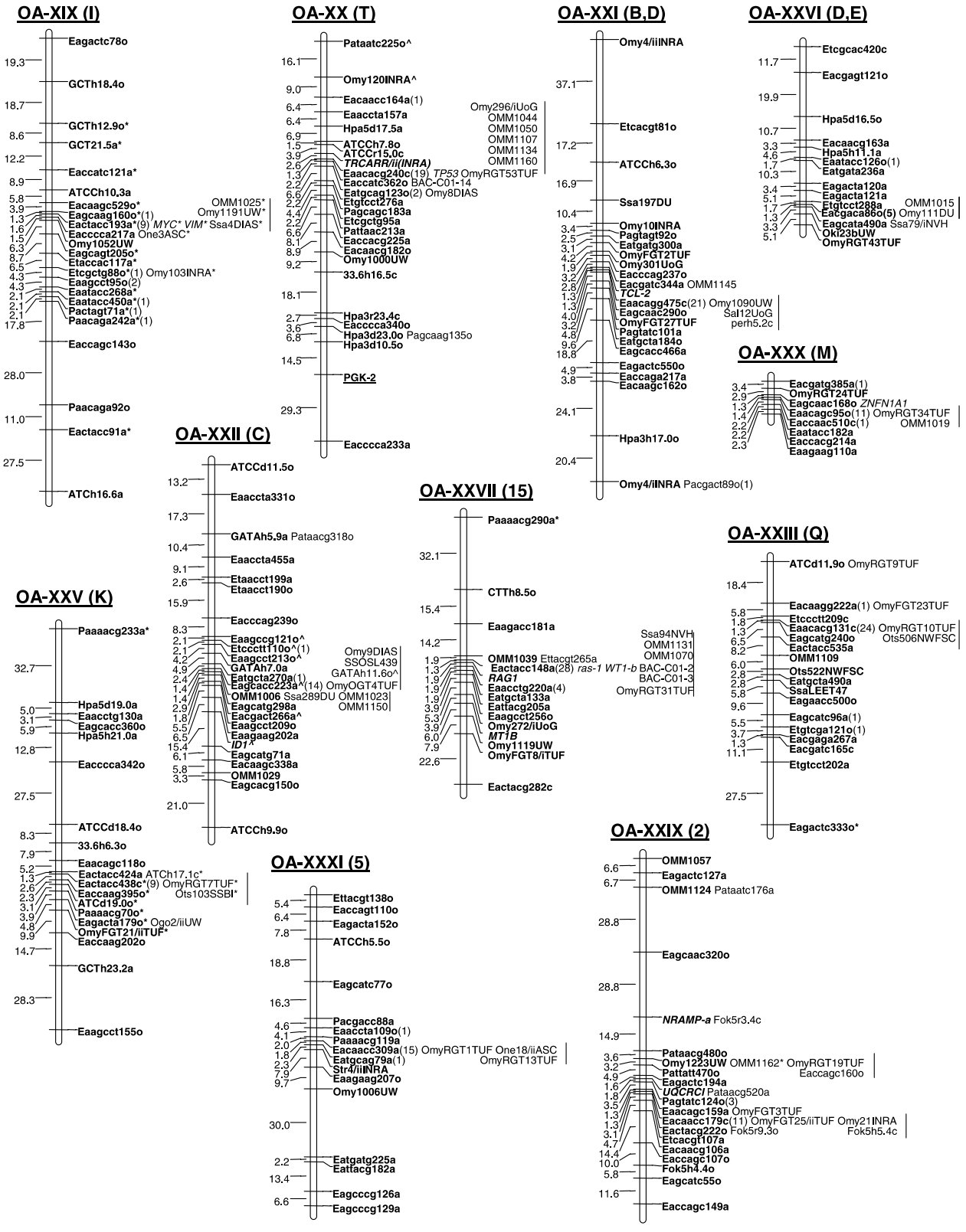

Figure 1 Continued.

distal arm away from the sex locus assuming that this cluster of markers represents the centromere. The Y-chromosomal DNA probe found in chinook salmon (*OtY1*; Devlin *et al*. 1994) is over 90% conserved in rainbow trout (Phillips unpublished data), but was not sex-linked in this rainbow trout cross. *OtY1* is found on OA-XVII. The fact that *OtY1* is not sex-linked in this cross agrees with previous findings on the lack of homology between the sex chromosomes of salmonid fishes, as observed with physical mapping of sex-linked loci in a variety of species (Iturra *et al*. 2001; Phillips *et al*. 2001). The localization of *GH1* to OA-IX and *GH2* to OA-II suggests that these linkage groups are homeologues. *TRCARR/i(INRA)* on OA-IX and *TRCARR/ii(INRA)* on OA-XX suggests that these two linkage groups are also homeologous. However, the mapping of *TRCARR*/ii(INRA) proximal to *GH1* does not support this putative homeology unless it can be established that a translocation event has occurred between these two marker positions on OA-IX.

Segregation distortion

All distorted markers on the linkage map (Fig. 1) have been checked for reliability, were found to be free of genotyping errors and ambiguity, and thus were retained. There was no significant difference in the proportions of distorted markers among the *Eco*RI AFLP, *Pst*I AFLP, microsatellite, gene, SINE and VNTR marker classes ($\chi^2 = 8.267$, d.f. $= 5$, $P = 0.142$). In most cases, markers with distorted segregation ratios are found together in blocks on linkage groups.

Synteny with other published rainbow trout maps

With allozymes in previous crosses: The addition of four allozymes and the DNA marker for the allozyme *SOD1* to the map has enabled us to identify syntenies with previous salmonid allozyme mapping efforts that include the composite salmonid map of May & Johnson (1990) and sex chromosome mapping of Allendorf *et al*. (1994). bGLUA (formerly HEX) and *SOD1* mapped to the same linkage group as sex in this map, which is consistent with previous linkage mapping in *O. mykiss* (May & Johnson 1990; Allendorf *et al*. 1994). sIDHP-1,2, found on OA-XVI, is syntenic with the composite allozyme map group 5. sMDH-B1,2, detected as a single locus and mapped to OA-VI, is syntenic with sMDH-B1 (formerly Mdh-3) and sMDH-B2 (formerly Mdh-4) on linkage groups 13 and 14, respectively, in the May & Johnson (1990) map. PGK-2 is not found on the composite allozyme map, but was cited by May & Johnson (1990) to occur approximately 40 cM from the centromere by gene-centromere mapping. This agrees with our results, which shows that PGK-2 is found distally on OA linkage group XX. Furthermore, data from the Sakamoto *et al*.'s (1999) map suggest that OA-XXIX is syntenic with May and Johnson's linkage group 2 due to the placement of sG3PDH-1 on this linkage group. OA-XXVII and OA-XVI may represent May and Johnson's pseudolinkage group V, as sIDHP-1,2 (formerly named IDH-3,4) maps to the latter linkage group and mIDHP-2 was reported to be linked with syntenic markers from OA-XXVII by Sakamoto *et al*. (2000).

With rainbow trout microsatellite map: At least two markers previously mapped in Sakamoto *et al*. (2000) were localized to each major linkage group for the OA doubled haploid progeny, enabling the establishment of synteny between the two rainbow trout maps. Homology between the designated linkage groups from this OA mapping family and the Sakamoto *et al*. (2000) mapping panel was unambiguously identified with the exceptions of linkage groups OA-IV, OA-V, OA-XVIII and OA-XXVIII (Table 4). The identification of a linkage group homologous to OA-XXVIII was not possible due to the lack of shared microsatellite markers, but this group is not likely a complete linkage group. The localization of *Ssa100NVH* to OA-V suggests that OA-V is homologous to linkage group H of Sakamoto *et al*. (2000). However, this marker is duplicated in the Sakamoto *et al*. (2000) mapping panel with one of the duplicates on linkage group H and the other on a newly identified linkage group (W; unpublished results). As a larger number of markers from group H share homology with linkage group OA-X, it is likely that OA-V is homologous to group W and homeologous to linkage group H of the Sakamoto *et al*. (2000) map. Homeologies identify linkage groups or segments of linkage groups with a shared ancestry from the ancient genome duplication that occurred within the salmonid lineage. Similarly, a putative duplicated marker of *Omy38DU* [on linkage group H in Sakamoto *et al*. (2000), map] appears to map to its homeologue, OA-XVIII, in the OA mapping panel. Linkage groups H and U are homeologous; thus OA-XVIII is more likely homologous to Sakamoto *et al*. (2000) linkage group U. For OA-IV, the localization of *Ssa72NVH* to this linkage group suggests synteny with linkage group K in Sakamoto *et al*. (2000). However, all other K markers are found together on OA-XXV, suggesting either a telomeric translocation of *Ssa72NVH* to OA-IV or pseudolinkage of this marker in the DH male. We believe that the localization of *Ssa72NVH* to OA-IV suggests that this linkage group may have homeology (rather than homology) to linkage group K of Sakamoto *et al*. (1999), but more syntenic markers on these groups is needed in order to confirm this.

Several OA linkage groups matched to more than one linkage group from Sakamoto *et al*. (2000). Based on the location of duplicated microsatellite markers, we believe that this reveals new putative linkage group homeologies. For example, cross assignments between markers on linkage groups P and E were made to OA-XI (Table 4), suggesting that the region containing *SSOSL32* in linkage groups P and E may be homeologous. *SSOSL32* maps adjacent to *OmyFGT24TUF* in the female map of Sakamoto *et al*. (2000).

Table 4 OA doubled haploid (DH) map syntenies with Sakamoto *et al*. (2000) outcross (OC) map. Note that XXVIII is not present in this table, as no markers are shared with that of Sakamoto *et al*. (2000).

Linkage group – DH (OA)	Linkage group – OC	Shared markers
I	18	*OmyFGT19TUF*, *Ots517NWFSC*
II	O_{ii}	*OmyFGT25/iTUF(2 + O_{ii})*[1], *Omy27DU*, *Ots521NWFSC*, *Ogo7/iiUW*, *OmyFGT18/iiTUF*, *Ogo7/iUW*
III	A	*Ogo2/iUW*, *Ogo1UW*
IV	?	*One6ASC(?)*, *Ssa72NVH(K)*
V	W[2]	*OmyFGT6TUF(?)*, *Ssa100NVH(H + W)*
VI	S	*FGF6(DIAS)*, *Ssa20·19*NUIG, *OmyFGT20TUF*
VII	R	*One1/iASC*, *One1/iiASC*, *OmyFGT26TUF*, *Omy7INRA*, *Ogo4UW*, *OmyRGT15/iTUF(?)*
VIII	N, J	*Ocl4UW(N)*, *OmyFGT12TUF(J)*
IX	O_i	*OmyFGT18/iTUF*, *TRCARR/i(INRA)*, *One14ASC*, *GH1(INRA)*, *OmyRGT30/iTUF*
X	H	*OmyRGT2TUF(H)*, *One10/iASC(H)*, *Ssa10NVH(H)*, *Ssa7/iNVH(?)*, *Omy7/iDIAS(?)*
XI	P, E	*Ocl1UW(P)*, *Ssa61NVH(?)*, *Omy7/iiDIAS(P)*, *SSOSL32(E)*, *Omy2DU(P)*
XII	F_i	*Ots100SSBI*, *Omy16DIAS*
XIII	N	*OmyJTUF*, *OmyRGT14TUF*
XIV	D	*Omy296/iiUoG*, *Ssa14DU*
XV	8	*Omy107INRA*, *Ots1BML*, *OmyRGT8TUF*, *OmyRGT23TUF*
XVI	F_{ii}, 5	*Omy77DU(F_{ii})*, *OmyOGT/iiTUF(F_{ii})*, *Str2INRA(F_{ii})*, *Ssa119/iiNVH(F_{ii})*, *IDH3,4(5)*
XVII	L	*Omy113INRA*, *Omy11DIAS*, *Ssa122NVH(?)*
XVIII	U	*OmyRGT12TUF(U)*, *Omy38DU(H)*, *Omy1DIAS(?)*
XIX	I[3]	*Ssa4DIAS*, *Omy103INRA*, *One3ASC*
XX	T	*Omy120INRA(?)*, *TRCARR/ii(INRA)*, *Omy8DIAS*, *Omy296/iTUF*
XXI	B, D	*Ssa197DU(B)*, *Sal12UoG(B)*, *OmyFGT27TUF(B)*, *Omy301UoG(B)*, *OmyFGT2TUF(B)*, *Omy10INRA(B)*, *Omy4/iiINRA(D)*, *Omy4/iINRA(?)*
XXII	C	*OmyOGT4TUF*, *Omy9DIAS*, *SSOSL439*, *Ssa289DU*
XXIII	Q	*OmyFGT23TUF*, *OmyRGT10TUF*, *Ots506NWFSC*, *OmyRGT9TUF(?)*, *SsaLEET47(?)*, *Ots522NWFSC*
XXIV	G	*One11ASC*, *Omy4DIAS*, *OmyRGT36TUF*, *One2ASC*, *Ssa57NVH*
XXV	K	*OmyRGT7TUF*, *Ogo2/iiUW*, *OmyFGT21/iiTUF*
XXVI	D, E	*OmyRGT43TUF(D)*, *Ssa79NVH(D + E)*, *Omy111DU(E)*
XXVII	15	*Ssa94NVH*, *ras-1(DIAS)*, *OmyRGT31TUF*, *Omy272/iUoG*, *OmyFGT8/iTUF*
XXIX	2	*OmyRGT19TUF*, *OmyFGT3TUF*, *Omy21INRA*, *OmyFGT25/iiTUF(2 + O_{ii})*
XXX	M	*OmyFGT24TUF*, *OmyRGT34TUF*
XXXI	5	*OmyRGT1TUF*, *OmyRGT13TUF*, *One18/iiASC*, *Str4/iiINRA*

[1]When more than two linkage groups are indicated these represent homeologous linkage groups identified by the duplicated marker polymorphisms. Where no parentheses are found, these markers are found on the OC linkage group indicated to have synteny with OA linkage group.

[2]Unpublished designations with the OC mapping families.

[3]Sakamoto *et al*. (2000) linkage groups I and V are now joined (unpublished data) and the ? indicate markers unassigned to a linkage group in Sakamoto *et al*. (2000) mapping panel.

As this latter marker has a gene-centromere distance of 46.4 cM (unpublished data), it is possible that a telomeric translocation may account for the synteny of this region with linkage group P. The homology of OA-XXVI to both linkage groups D and E of the Sakamoto *et al*. (2000) mapping panel can be explained by the homeology of marker *Ssa79NVH* to other linkage group D markers. This marker was recognized as being duplicated in the Sakamoto *et al*. (1999) study, but only one of the duplicated pair was polymorphic and was mapped to linkage group E. The marker mapped for the doubled haploid family is more likely the duplicate of *Ssa79NVH*. The present localization of this marker to linkage group E in Sakamoto *et al*. (2000) and the duplicate mapped to OA-XXVI (homologous to linkage group D) suggest that D and E may be homeologous. In addition, we believe that *Omy38DU* mapped in the OA cross is the duplicate of the *Omy38DU* mapped in Sakamoto *et al*. (2000) mapping panel. As the duplicate marker *OmyCosBTUF* indicates that OA-X and OA-XVIII may be homeologous, the placement of *Omy38DU* to OA-XVIII suggests that the duplicate marker has been genotyped in this family.

Based on the data herein, the following putative homeologies are inferred among the linkage groups identified in the OA doubled haploid mapping family: II and IX, III and XXV, X and XI, XIV and XX, IX and XX, II and XXIX (Table 5). Syntenies established between this map and the Sakamoto *et al*. (2000) map, and data therein further suggest potential homeologies among IV and XXV, X and XVIII, XII and XVI, XVII and XXII, XXIII and XXIV, XXVII and XXXI. The localization of one marker from linkage group 5

Table 5 Inferred homeologies among OA linkage groups from duplicated microsatellite and known gene markers.

OA linkage group	Homeologue	Markers conferring homeology
I		
II	IX	*GH1, GH2; OmyFGT18/iiTUF, OmyFGT18/iTUF*
	XXIX	*OmyFGT25/iTUF, OmyFGT25/iiTUF*
III	XXV	*Ogo2/iUW, Ogo2/iiUW*
IV	XXV[1]	*Ssa72NVH*
V		
VI		
VII		
VIII		
IX	II	*GH1, GH2; OmyFGT18/iiTUF, OmyFGT18/iTUF*
	XX	*TRCARR/i(INRA), TRCARR/ii(INRA)*
X	XI	*Omy7/iDIAS, Omy7/iiDIAS*
	XVIII[1]	*OmyCosBTUF*[1]
XI	X	*Omy7/iiDIAS, Omy7/iDIAS*
XII	XVI[1]	*Omy3INRA*[1], *OmyOGT5TUF*[1], *OmyRT10TUF*[1]
XIII		
XIV	XX	*Omy296/iiUoG, Omy296/iUoG*
XV		
XVI	XII[1]	*Omy3INRA*[1], *OmyOGT5TUF*[1], *OmyRT10TUF*[1]
XVII	XXII[1]	*OmyRGT6TUF*[1]
XVIII	X[1]	*OmyCosBTUF*[1]
XIX		
XX	IX	*TRCARR/i(INRA), TRCARR/ii(INRA)*
	XIV	*Omy296/iUoG, Omy296/iiUoG*
XXI		
XXII	XVII[1]	*OmyRGT6TUF*[1]
XXIII	XXIV[1]	*Omy27INRA*[1]
XXIV	XXIII[1]	*Omy27INRA*[1]
XXV	III	*Ogo2/iUW, Ogo2/iiUW*
XXVI		
XXVII	XXXI[1]	*One18ASC*[1], *OmyFGT8TUF*[1], *Omy272UoG*[1]
XXIX		
XXX		
XXXI	XXVII[1]	*One18ASC*[1], *OmyFGT8TUF*[1], *Omy272UoG*[1]

[1]Inferred jointly from syntenies with the Sakamoto *et al*. (2000) map and homeologies detected therein.

of Sakamoto *et al*. (2000) (i.e. sIDHP-1,2) to OA-XVI in combination with a larger number of markers from linkage group F_{ii} (Table 3) is unexpected. As this marker is more telomerically located in the Sakamoto *et al*. (2000) map, it is possible that a telomeric translocation has occurred. However, sIDHP-1,2 was only localized to linkage group 5 in the male mapping parent of Sakamoto *et al*. (2000), and may therefore represent a pseudolinkage in the male. If this is true then it suggests that OA-XXXI and one arm of OA-XVI are also homeologous. A telomeric translocation may also explain the linkage of marker *Omy4/iiINRA (*linkage group D) to a larger number of intercalary markers from linkage group B (localized to OA-XXI). In the OA mapping panel, markers from Sakamoto *et al*. (2000) linkage groups N and J are found together on OA-VIII. Homeologous regions for linkage groups N and J were not identified in Sakamoto *et al*. (2000) study, and this most likely represents a difference in the chromosome arrangements between the two crosses. The current affinities suggest that OA-VIII and OA-XIII may be homeologs.

Discussion

This consolidated genetic linkage map is the most dense map published for any salmonid species and provides information on the syntenies and homeologies among linkage groups from mapping efforts in doubled haploid (Young *et al*. 1998) and outcrossed (Sakamoto *et al*. 2000) rainbow trout families. The sex chromosomes have also been mapped with greater resolution. Young *et al*. (1998) reported 31 major linkage groups with the initial map produced using these doubled haploids. The OSU line has a haploid chromosome number of 30, while Arlee has a haploid chromosome number of 32 (Ristow *et al*. 1998). Linkage mapping in this OSU × Arlee cross should result in 30 major linkage groups, as four acrocentric chromosomes

from Arlee would pair with two metacentric chromosomes from OSU. With the addition of more than 900 markers, we identified 30 major linkage groups, which agrees with the number expected for this cross. The 'extra' linkage group from the original map (OA-XXVIII) did not benefit from the addition of markers, lacks the clustering characteristic of the other major linkage groups, and is more likely not a representative of a complete physical chromosome. The inability to link some of the small groups to the major framework groups could be an artifact of residual tetrasomy that results from the ancient genome duplication that occurred early in the evolutionary history of the salmonids. A greater than expected number of recombinants might be observed between physically linked loci if telomeric ends are pairing with both homeologous and homologous chromosome arms. In this case, recombination can occur between homeologs and homologs, thereby skewing the recombination rates that would be observed with strict homologous pairing (Johnson *et al.* 1987; Sakamoto *et al.* 2000). All but one linkage group, OA-IV, were matched to linkage groups identified in the outcrossed mapping panel of Sakamoto *et al.* (2000).

All marker classes except VNTR loci had greater densities in the central regions of the linkage groups. Research in other species has suggested that recombination is suppressed in centromeric regions (Keim *et al.* 1997) and we believe that the large clusters of markers on each linkage group represent the centromeres. The significant marker clustering is not specific to marker type in this cross. In pink salmon, Lindner *et al.* (2000) observed that AFLP markers exhibited greater densities and clustering in centromeric regions of chromosomes, while microsatellite and VNTR markers were distributed more towards the telomeres of chromosomes. However, in this cross, all marker classes except VNTRs were found at greater densities in the intercalary regions of the linkage groups. Young *et al.* (1999) observed a significant clustering of *Eco*RI AFLP markers relative to *Pst*I AFLP in the soya bean genome – a result of CG methylation patterns. This AFLP clustering difference was not observed in our doubled haploid mapping family, suggesting that alternate patterns or lower rates of methylation exist in rainbow trout. We believe that the notable clustering of all marker types in the middle regions of linkage groups is not necessarily characteristic of marker type, but characteristic of the recombination patterns observed during meiosis in male salmonids.

The clustering of markers in central regions of linkage groups has been noted in the males of many species (Sakamoto *et al.* 2000 and references therein; Singer *et al.* 2002 and references therein), but the degree of recombination suppression may be much greater in male salmonids. In fact, Sakamoto *et al.* (2000) have reported a 3.25 : 1 female to male linkage map distance ratio, which is higher than that reported for any other vertebrate. This phenomenon is most likely due to the residual tetrasomic segregation patterns that result from the formation of multivalents during meiosis in male salmonids (Wright *et al.* 1983; Johnson *et al.* 1987; Allendorf & Danzmann 1997). In this doubled haploid map, produced from male hybrid clones, the inflated distances in chromosome arms and exaggerated clustering of markers in central regions is likely due to the multivalent pairing of chromosomes during meiosis, and the structural constraints imposed by homeologous pairing that repress recombination in central areas of the chromosomes.

The addition of type I markers to the map affords limited information for the study of syntenic blocks between this rainbow trout cross and other vertebrate organisms. *RAG1* and *WT1-b* on OA-XXVII are syntenic on chromosome 25 in zebrafish and on chromosome 11 in humans (Woods *et al.* 2000). Evaluation of conserved syntenic blocks for other linkage groups is not yet possible, as many of the OA linkage groups do not have more than one type I locus currently mapped.

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